ANTIDIABETIC POTENTIAL OF COMBINATORIAL HERBAL FORMULATION IN HIGH FAT DIET INDUCED TYPE 2 DIABETIC RATS - A MOLECULAR APPROACH

SYNOPSIS

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INTRODUCTION:

Diabetes Mellitus is a chronic disease characterized by hyperglycemia and is categorized into two types: Type I Diabetes Mellitus (T1DM) and Type II Diabetes Mellitus (T2DM). In T1DM, β-cells of the pancreas are damaged, leading to a decreased insulin supply to the circulation. Patients will be fully dependent on exogenous insulin administration for existence. Contrarily, T2DM has been observed in majority of diabetic patients (85%) and results in peripheral insulin resistance, thereby results in decreased insulin sensitivity to the skeletal muscles, adipose tissues and liver (Forbes JM and Cooper ME. 2013). Another category of diabetes can also be categorized in pregnant women without previously diagnosed diabetes, known as gestational diabetes mellitus. Factors such as aging, obesity, physical inactivity, population growth and urbanization can gradually leads to steady increase in the number of patients with diabetes. In year 2000, prevalence of diabetes worldwide among adults is estimated to be approximately 171 million, 2021 whereas the number has been increased to 422 million (approximately 1 in every 11 people) in 2014 (Li W *et al*., 2017). The prevalence of diabetes in the world is expected to be doubled to approximately 366 million in year 2030 due to demographic changes in people of more than 65 years old and most importantly, adaptation of sedentary life style by the people in the urban areas of the world (Sarah wild *et al*., 2004). If this disease left untreated it can lead to acute fatal complications including diabetic keto acidosis and coma due to exceptional increase in blood glucose. Additional dreadful consequences of diabetes include vascular complications due to damage of the vessels for high glucose level, may result in macro vascular and micro vascular disorders. Consequences of micro vascular complications are retinopathy, neuropathy, etc., whereas, macro vascular complications lead to cardiovascular complications. Other complications for chronic diabetic conditions include dementia, sexual dysfunction, depression and lower-limbamputations (Forbes JM and Cooper ME. 2013).Different categories of antidiabetic medications are there in the market for the remedial action, which includes insulin analogues, sulphonylureas, biguanides, dipeptidyl peptidase-4 inhibitors,

thiazolidiones, a-glucosidase inhibitors etc, where the mechanism of counteracting this increased glucose level is different for different categories (Li W *et al*., 2017). However, long term treatment and side effect of the available hypoglycemic medications leading towards huge demand for efficacious, decreased side effects and affordable agents for the treatment of diabetic condition.

The conventional drugs are used to treat diabetes by improving insulin sensitivity, increasing insulin production and decreasing the amount of glucose in blood. The adverse effect of drug treatment are not always satisfactory in maintaining normal levels of blood glucose and this view many medicinal plants have been provided a potential source of antidiabetic principle which are widely used for the treatment of diabetes mellitus in various traditional system of medicine worldwide and many of them are known to be effective against diabetes. The hypoglycemic effect of pharmacologically active component of plant decrease the effect on α-amylase and various direct and indirect effects of different blood parameters responsible for development of diabetes (Murali Mohan N. 2006). A large number of antidiabetic medicines are available in the pharmaceutical market for diabetes and its related complications; however, currently no effective therapy is available to cure the disease. However, due to unwanted side effects the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes (Moller DE. 2001). In recent years, there has been an increased inclination towards the herbal formulations due to the trend towards the natural sources that are with minimal side effects. The mixture of several crude extracts, when used in formulation enhances the beneficial effects through synergistic amplification and diminishes any possible adverse effects and offers advantage over a single isolated ingredient.

Flower of Cassia Auriculata:

Cassia auriculata L. also known as “avaram” in Tamil language is a shrub belongs to the Caesalpiniaceae family. It is one of the principle constituents of ‘Avaarai panchaga chooranam’- an Indian herbal formulation used in the treatment of diabetes to control the blood sugar level

(Brahmachari HB and Augsti KT. 1961). The shrub is especially famous for its attractive yellow flowers which are used in the treatment of skin disorders and body odour. It is widely used in traditional medicine for rheumatism, conjunctivitis and diabetes. It has many medicinal properties. Its bark is used as an astringent, leaves and fruits anthelminthic, seeds used to treat in eye troubles and root employed in skin diseases (Siva, R. and Krishnamurthy, K.V. 2005). It is also used for the treatment of ulcers, leprosy and liver disease (Kumar, R.S *et al.,* 2002). The antidiabetic hypolipidemic (Umadevi, P *et al*., 2006), antioxidant, hepatoprotective (Kumar, R.S *et al*., 2003) Antifungal (Deborah KB runyoro *et al*., 2006), Antibacterial (Abo *et al.*, 2000) Hepatoprotective activity (Joy *et al*, 2012) Antidiabetic activity (Jalalpure *et al*., 2004). Effect of Cassia auriculata have been reported. It was also observed that flower and leaf extract of Cassia shown to have antipyretic activity.

Leaves of Glymnema Sylvestre:

Gymnema sylvestre is one of the important anti-diabetic medicinal plant, there is a growing demand for G. sylvestre leaves in the pharmaceutical trade. The “destroyer of sugar” is a traditionally used term for Gymnema sylvestre because chewing the leaves will abolish the taste of sweetness. Gymnema sylvestre is a slow growing, perennial medicinal woody climber found in central and peninsular India. G. sylvestre leaves contain triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are gymnemic acids and gymnema saponins, while dammarenesaponins aregymnemasides. Besides this, other plant constituents are flavones, anthraquinones, hentriacontane, pentatriacontane, I and J-chlorophylls,phytin, resins, d-quercitol, tartaric acid, formic acid,butyric acid, lupeol, J-amyrin related glycosides and stigmasterol. The plant extract also tests positive for alkaloids. Leaves of this species yield acidic glycosides and anthroquinones and their derivatives (Dateo G.P and Long L. 1973). Among these bioactive compounds of Gymnema sylvestre, gymnemic acids have anti-diabetic, anti-saccharineand anti-inflammatory activities (Masayuki Y, *et al*., 1997) Traditionally it was recommended for stomach

problems, constipation, liver disease but the recent studies have shown that the extract of Gymnema sylvestre is useful in controlling blood sugar to treat type-II diabetes (NIDDM). When Gymnema leaf extract is administered to a diabetic patient, there is stimulation of the pancreas by virtue of which there is an increase in insulin release (Persaud S J, *et al.*, 1999).

CinnamomumZeylanicum:

Cinnamon is a common spice used by different cultures around the world for several centuries, also used as a food antioxidant and to enhance food palatability. It is obtained from the inner bark of trees from the genus cinnamomum, a tropical evergreen plant that has two main varieties; Cinnamomum zeylanicum (CZ) and Cinnamon cassia (CC). In addition to its culinary uses, in native Ayurvedic medicine Cinnamon is considered a remedy for respiratory, digestive and gynaecological ailments, in-vitro and in-vivo studies in animals and humans from different parts of the world have demonstrated numerous beneficial health effects of CZ, such as anti-inflammatory properties, anti-microbial activity, reducing cardiovascular disease, boosting cognitive function and reducing risk of colonic cancer, on the effects of CZ extracts on diabetes demonstrates numerous beneficial effects both in-vitro and in-vivo. In-vitro CZ has demonstrated a potential for reducing post-prandial intestinal glucose absorption and stimulating glucose metabolism and glycogen synthesis, in addition to stimulate insulin release and potentiating insulin receptor activity (Priyanga Ranasinghe *et al.,* 2013; Ranasinghe P *et al.,* 2012). Other studies showed that aqueous extracts from cinnamon have been shown to increase in vitro glucose uptake and glycogen synthesis as well as to increase phosphorylation of the insulin receptor. Also cinnamon extracts are likely to aid in triggering the insulin cascade system. These data suggest that cinnamon prevents the development of insulin resistance and aqueous extract of cinnamon has also been shown to improve insulin sensitivity in humans (Khalse M. A *et al*., 2013; Bolin Qin *et al*., 2010).

Seeds of Syzygium Cumini:

Syzygium cumini, commonly known as “jamun” (family Myrtaceae), is one of them. Syzygium cumini has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties. S. cumini seeds are rich source of phytochemicals and researchers are taking keen interest in exploring its role in different diseases. Flavonoids such as rutin and quercetin, well known antioxidants and mono terpenoids are present in the seeds, which accounts for the scavenging of free radicals and protective effect on antioxidant enzyme (The Wealth of India .1982).S. cumini and its parts have been used as an alternative and complementary medicine to regulate diabetes all over theworld. Each and every part of the plant stem bark, leaves, fruit and seed shows its prospective role in regulating different diseases, as diabetes, cancer, mouth ulcer, colicdiarrhea, dysentery, piles, pimples and indigestion (Jain SK. 1991). The bark is acrid, sweet digestive astringent helps in blood purification and used for the treatment of the sore throat. The fruit is used to remove bad smell from the mouth, biliousness, stomachic, astringent, diuretic, anti-diabetic (Nadkarni KM. 1976). The seed extract is used to treat cough, cold, fever, skin rashesand mouth throat, intestines and verymuch effective as anti-oxidant, anti-inflammatory, antimicrobial, antibacterial anti-HIV, anti-fungal, anti-diarrheal, anti-fertility, gastro-protective anti ulcerogenic and radioprotective activities and many more (Sagrawat H *et al.*, 2006). The efficacy of S.cumini seeds for the treatment of diabetes has been tested inseveral clinical and experimental studies proposing various mode of action.

Trigonella Foenum Graecum (Fenugreek):

Trigonella foenum graecum (fenugreek) is a self-pollinating plant that grows annually through out regions of North and South America, Africa, Australia, Asia and Europe and is used as a daily ingredient in Africa, Asia, and the Mediterranean (Liu Y *et al*., 2012). Fenugreek can be consumed as a powder, dried seed, fried, or raw. Fenugreek seeds and leaves consist of vitamins,

minerals, fibers, saponins, alkaloids, flavonoids, carbohydrates, proteins and lipids, iron, and calcium (Goyal S *et al.*, 2016). Fenugreek seeds also have a high volume of antioxidants. These phytochemical and chemical compounds serve as protective agents for diabetes mellitus and hypoglycemia, liver, and kidney disorders. Various studies have shown the positive effect of fenugreek seed extract in curbing symptoms of diabetes mellitus, including glucose absorption and insulin resistance.

SCOPE OF THE PRESENT STUDY:

Liver is a major insulin responsive target organs which clear glucose in blood and insulin resistant. Therefore we focus on liver to study the glucose clearance and insulin resistant in high fat diet induced type 2 diabetic rats by using the poly herbal combination.

Mixture of several crude extracts, when used in formulation it enhances the beneficial effects through synergistic amplification and diminishes any possible adverse effects and offer advantage over a single isolated ingredient. Therefore, we plan to study polyherbal formulation against type 2 diabetic adult male wistar rats.

AIM:

To identify the antidiabetic potential of combinatorial herbal formulation in high fat diet induced type 2 diabetic male wistar rats.

OBJECTIVES

To identify the effects of Polyherbal on

- Glucose and insulin tolerances, serum insulin and serum lipid profile, enzymatic and non-enzymatic antioxidants, oxidative stress parameters in type 2 diabetic adult male wistar rat.
- Insulin signaling molecules (IR, IRS, Akt, SREBP, AS160 & GLUT2) glucose uptake and oxidation in liver of type 2 diabetic adult male wistar rat.

MATERIALS AND METHODS

Animals

The experimental study was approved by the institutional animal ethics committee (IAEC No: 08/2016 dated 23.01.2016) as per the National Guidelines and Protocols. The present experimental studies have been approved by the institutional animal ethical committee which is sanctioned 36 adult male wistar albino rats. Healthy adult male Wistar albino rats (150-180 days old weighing 180-200g) were obtained and maintained in clean polypropylene cages at the Central Animal House Facility, Sri Muthukumaran Medical College hospital & Research Institute. They were fed with standard rat pelleted diet (Lipton India, Mumbai, India), and clean drinking water was made available ad libitum.

INDUCTION OF TYPE 2 DIABETES:

Rats were fed high-fat diet containing cholesterol 3%, cholic acid 1%, coconut oil 30% and standard rat feed 66% along with this 30% sucrose through drinking water for the period of 60 days. On the 60th day of feeding, after overnight fasting, blood glucose was checked and the rats which have blood glucose above 120 mg/dl will be chosen as type 2 diabetic rats. Treatment would be started on the next day after confirmation of type 2 diabetes and this would be considered as 1st day of treatment and it will be continued for 45 days. The rat were fed with high fat diet during this period (Nampurath *et al*. 2008).

Sucrose is a disaccharide composed of the monosaccharides glucose and fructose. In humans and other mammals, sucrose is broken down into its constituent monosaccharides by sucrose or isomaltase, glycoside hydrolases, which are located in the membrane of the microvilli lining the duodenum. The resulting glucose and fructose molecules are then rapidly absorbed into the bloodstream. Excessive glucose will be stored as glycogen. Fructose is more lipogenic than glucose or starches. It induces moderate obesity and several adverse metabolic effects, including

hypertriglyceridemia, hyperinsulinemia and hypertension in rodents. Fructose bypasses the phosphofructokinase regulatory step and enters the pathway of glycolysis or gluconeogenesis at the triose phosphate level, resulting in increased hepatic triglyceride production. Excess storage of saturated fat in white adipose tissue causes hypertrophy and hyperplasia of adipocytes, which exhibit attenuated insulin signaling due to their production and release of saturated fatty acids. These adipocytes recruit macrophages to white adipose tissue and together with them, initiate proinflammatory response. Proinflammatory factors and saturated fatty acids secreted into the blood stream from white adipose tissue impair insulin signaling in non-adipose tissues, which causes whole body insulin resistance (Funaki, 2009).

PREPARATION OF POLY HERBAL

Cassia auriculata (Flowers), Gymnema sylvestre (Leaves), Trigonella foenum graecum seed (Fenugreek), Syzygium cumini seed (L.) and Cinnamomum bark were purchased from Local market at Chennai. Above mentions drugs will be mixed in a define ratio.

DOSE FIXATION STUDY

The optimal dose of Polyherbal drug (PHD) was fixed on the basis of following dose fixation study. Rats were randomly divided into 6 groups each consisting of 3 rats each. Group I: Control, Group II: Diabetic rats, Group III: Diabetic rat treated with Polyherbal drug 100 mg/kg b.wt/day orally for 45 days, Group IV: Diabetic rat treated with Polyherbal drug 250 mg/kg b.wt/day orally for 45 days, Group V: Diabetic rat treated with Polyherbal drug 500 mg/kg b.wt/day orally for 45 days, Group VI: Diabetic rat treated with Metformin 50 mg/kg b.wt/day orally for 45 days.

During the experimental period, blood samples were collected analysed. At the end of experimental period, rats were anaesthetized with Sodium Thiopentone (40mg/Kg b.wt). Blood was collected through cardiac puncture sera was separated for the determination of liver and kidney

function markers and stored at -80° C. Isotonic Nacl solution was perfused through the left ventricle to clear blood from the organs.

Polyherbal drug with aqueous extract at the dose of 100 mg/kg b.wt did not reach the normal levels of kidney function markers (Urea, Creatinine and blood urea nitrogen), liver function markers (ALP, ALT and AST) of control groups. Whereas 250 and 500 mg/kg b.wt doses of Polyherbal drug treatment for 45 days significantly normalized the liver and kidney function markers to that of the control group. Based on this, the 250 mg/kg b.wt dose was selected as the optimal dose for further analysis.

EXPERIMENTAL DESIGN

The following experimental design was framed and accordingly the rats were subjected to treatment for a period of 45 days. Healthy adult male wistar rats were divided into the following groups.

Group I	Control rats
Group II	Diabetic rats (Type -2)
Group III	Diabetic rats (Type -2) treated with Polyherbal drug (250 mg/ kg b.wt/day, orally for 45 days).
Group IV	Diabetic rats (Type -2) treated with Metformin (50 mg/ kg b.wt/day, orally for 45 days).
Group V	Control rats treated with Polyherbal drug (250 mg/ kg b.wt/day, orally for 45 days).

At the end of 45 days, the animals will be subjected to anaesthesia with sodium thiopentone (40mg/kg b.wt) blood was collected and animals were perfused with normal saline. The liver from control and experimental animals were dissected out for the assessment of various parameters.

BIOCHEMICAL ESTIMATION:

Fasting blood glucose (FBG)

Blood glucose will be estimated using On-Call plus glucose test strips (ACON laboratories Inc., USA) after overnight fasting. Blood will be collected by nicking the tip of the rat tail and results will be expressed as mg/dl.

Oral Glucose Tolerance Test (OGTT)

For oral glucose tolerance test, animals will be fasted overnight and blood glucose will be estimated using On-Call plus blood glucose test strips at various time periods (60, 120 and 180 min) after giving the oral glucose load (10 ml/kg; 50% w/v). Blood glucose value before giving glucose load will be considered as 0 minute value. Results will be expressed as mg/dl.

Insulin Tolerance Test (ITT)

This test will be performed on random-fed rats. Rats will be injected with insulin (0.75 U/kg) in~0.1ml 0.9% saline intraperitoneally. A drop of blood (5µl) will be taken from the tail vein before the injection of insulin and after 15, 30, 45 and 60 min for the determination of blood glucose with a glucometer. Results will be expressed as mg/dl

Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI)

HOMA-IR will be calculated using the formula (Fasting blood glucose X fasting serum insulin / 405) as per the method of Mathews *et al*. (1985) and QUICKI will be calculated using the formula 1/ (log fasting serum insulin + log fasting blood glucose) as per the method of Katz *et al*. (2000).

Liver and kidney function markers

Liver function markers (ALT, AST and ALP) and Kidney function markers (urea, creatinine, bilirubin, blood urea nitrogen) were measured using biochemical-assay kits procured from Spin react, Spain using Semi-Automated Biochemistry Analyser System (Cora lab 3000).

Serum lipid profile

Serum cholesterol (CHO), triglyceride (TG), low-density lipoproteins (LDL), High-density lipoproteins (HDL) and free fatty acid (FFA) were assessed using assay kits purchased from spin react, Spain.

Lipid peroxidation and Reactive oxygen species

Lipid peroxidation (LPO) was measured by a previously published method of Devasagayam and Tarachand (1987). Hydrogen peroxide (H_2O_2) generation was assessed by the spectrophotometric method of Pick and Keisari (1981). Hydroxyl radical (OH*) production was quantified by the method of Puntarulo and Cederbaum (1998).

Antioxidant enzymes

Superoxide dismutase (SOD) activity was assessed by the method of Marklund and Marklund (1974). Catalase activity (CAT) was assessed as per the method of Sinha (1972). Glutathione peroxidase (GPx) levels were assessed by method of Rotruck *et al*. (1973). Glutathione-S-transferase (GST) activity was assessed by the method of Habig *et al*. (1974). Glutathione reductase (GR) was assessed as per the method of Staal *et al*. (1969) and reduced glutathione (GSH) levels were measured by the method of Moron *et al* (1979).

Determination of glycoproteins

The plasma and tissue hexose content was estimated by the method of Niebes (1972). Sialic acid in plasma and tissues were estimated by the method of Warren (1959) and hexosamine by the method of Wagner (1979). Fucose was estimated by the method of Dische and Shettles (1948).

Histological evaluation

For histological examination, tissues (Liver and pancreas) were excised and rinsed with ice-cold saline to remove blood and debris adhering to tissues. The tissues were then fixed in 10% formalin for 24 h. After dehydration through a graded andseries of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Sections were cut into 5 µm thickness and stained with haematoxylin and Eosin. After dehydration and cleaning, the sections were mounted and observed under light microscope (Nikon, Eclipse Ti, Japan) for histopathological analysis.

GENE EXPRESSION ANALYSIS:

mRNA expression of IR, IRS, Akt, SREBP, AS160 & GLUT2.

Total RNA was isolated from control and experimental samples using TRIR kit (Total RNA Isolation Reagent) from Invitrogen. Briefly, 100 mg fresh tissue was homogenized with 1 ml TRIR and the RNA was isolated according to Fourney *et al*. (1988). The purity of RNA obtained was 1.8. The yield of RNA is expressed in microgram (µg).

cDNA conversion and Real-time RT-PCR:

Total RNA was used for the synthesis of cDNA. The RT kit used was from Eurogentec (Seraing, Belgium). For this, 2 µg of total RNA was added to 10X reaction buffer, 25 mM MgCl2, 2.5 mMdNTPs, OligodT, RNaseinhibitor, Euro script reverse transcriptase enzyme and the final volume was made up to 20 µl with RNase free water. The tubes were kept in the thermocycler programmed as initiation step at 25°C for 10 min, reverse transcriptase step at 48°C for 30 min, inactivation of RT enzyme step at 95°C for 5 min.

The reaction mixture was prepared by adding all the components (2x reaction buffer (Takara sybr green mastermix), forward and reverse primers and water together (45 µl) except the cDNA templates of the target gene and house-keeping gene, β-actin. It is mixed thoroughly and spin down. About 5 µl of template cDNA for samples, 5 µl of control DNA for positive control and 5 µl of water

for negative control were taken in separate PCR vials. Then 45 µl of reaction mixture was added into the reaction vial and the reaction was set up for 40 cycles (95 ℃ for 5 min, 95°C for 5 seconds, 190 60°C for 20 seconds and 72°C for 40 seconds).The results obtained were plotted on a graph 191 by the PCR machine and an amplification curve and melt curve analysis were generated from 192 which relative quantification was calculated.

PROTEIN EXPRESSION ANALYSIS:

Preparation of tissue lysate

Procedure

Tissue was first cleaned of all visible fat, nerve and blood vessels and minced in Radio Immuno Precipitation Assay buffer (RIPA buffer). The minced liver was homogenized in RIPA buffer (1ml for 100mg) using homogenizer (Pro scientific, UK) at a precise low setting on ice. The homogenate was centrifuged at 1,300 x g for 10min at 4ºC. The supernatant was centrifuged at 12,000 x g for 15min at 4°C. The resultant supernatant was sampled as a total protein for the western blot analysis of IR, IRS, Akt, SREBP, AS160 and GLUT2 then the protein concentration was estimated (Lowry *et al.*,1951) using BSA (Bovine Serum Albumin) as a standard.

Western blot analysis

The lysate proteins (50 µg/lane) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% gel) and transferred by electroblotting to polyvinylidenedifluoride (PVDF) membrane (Bio-Rad Laboratories Inc.). The membranes 158 were blocked with 5% non-fat dry milk and probed with the primary antibodies (which were diluted to 1:1000). Following incubation, the blot was washed for three times (5 min each 160 with Tris-buffered saline containing Tween-20 (TBS-T)). After washing with TBS-T, the membranes were incubated for 1 hour with horse radish peroxidase-conjugated rabbit-anti mouse or goat-anti-rabbit antibodies (which were diluted to

1:5000) (GeNei, Bangalore, India). The specific signals were detected with an enhanced chemi luminescence detection system (Thermo Fisher scientific Inc, USA). The protein bands were captured using Chemidoc and quantified by Quantity One image analysis system Bio-Rad Laboratories, CA. Later the membranes were incubated in stripping buffer at 50°C for 30 min. After this the membrane was reprobed using a β-actin antibody (1:5000). As the invariant control, the present study used rat β-actin.

STATISTICAL ANALYSIS

The data's obtained were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the control and treatment groups using a computer based software (Graph Pad Prism version 5). In Duncan's test, the significance was considered at the level of $p<0.05$.

RESULTS

Dose dependent effect of Poly herbal drug in body weight, food intake

The body weight and food intake of the animals was significantly reduced due to diabetes. Treatment with of Polyherbal drug at the dosage of 100 mg/kg b.wt did not show any adverse effect in control and experimental groups. Whereas 250 and 500 mg/kg b.wt doses treatment significantly normalized the liver function markers and improved the body weight and food intake which altered by diabetes induction.

Dose dependent effects of Polyherbal drug in blood glucose, insulin, Hb and HbA1c.

The levels of glucose and HbA1c were significantly increased in diabetic rats, whereas the levels of insulin and Hb were significantly decreased in the diabetic rats when compared with control rats. Treatment with Polyherbal drug at the dosage of 100 mg/kg b.wt did not show any effective reduction on the elevated levels of glucose and HbA1c and could not increase the levels of insulin and haemoglobin. Whereas 250 and 500 mg/kg b.wt doses treatment significantly normalized the levels of glucose, HbA1c, insulin and Hb when compared with control rats.

Effects of Poly herbal drug on blood glucose, insulin, HOMA-IR, Hb and HbA1c in High Fat Diet induced type 2 diabetic rats.

Diabetes induced rats showed elevated levels of glucose, HOMA-IR and HbA1c were significantly increased, whereas the levels of insulin and Hb were significantly decreased in the diabetic rats when compared with control rats. Treatment with Polyherbal drug or metformin reversed these values to near normal in diabetic rats. Oral administration of Polyherbal drug to normoglycemic rats showed no significant changes in the above-said parameters.

Effect of Polyherbal drug on serum amino transferases activities and urea and creatinine levels

The activities of AST, ALT and the levels of serum urea and creatinine were increased significantly in diabetic rats when compared with control animals. Upon Polyherbal drug and metformin administrations, the levels were significantly reverted back to near normal levels when compared with untreated diabetic rats.

Effect of Polyherbal drug on serum lipid profile

The levels of total cholesterol, triglycerides, VLDL and LDL in plasma were increased significantly, whereas the level of HDL was decreased significantly in high fat- induced diabetic rats when compared with control rats. However, on treatment with Polyherbal drug, the levels of the above-said parameters were brought back to near normal levels compared to untreated diabetic control rats.

Polyherbal drug treatment modulates the oxidative stress markers

Compared to control, the hydrogen peroxide (H_2O_2), hydroxyl radical (OH*) and lipid peroxidation (LPO) levels were significantly increased in diabetic rats. Upon the treatment of Poly herbal drug the levels were significantly decreased.

Effect of Polyherbal drug on enzymic and non-enzymic antioxidants levels in serum and liver

Levels of LPO, enzymic antioxidants (SOD, CAT, GPx and GR) and non-enzymic antioxidants activities were decreased in untreated groups of rats. Treatment groups of rats showed increased activities of enzymic and non-enzymic antioxidants which were decreased high fat diet induced rats when compared with their control rats

Effects of Poly herbal drug on glycoprotein components

When compare to normal rats, the levels of Glycoproteins (hexose, hexosamine, fucose, and sialic acid) were significantly changed in plasma and tissue of HFD induced diabetic rats. On 45 days oral administration of poly herbal drug and metformin the levels of hexose, hexosamine, fucose, and sialic acid were significantly decresed in plasma. The levels of hexose, hexosamine, and fucose were significantly increased whereas the levels of sialic acid were significantly decreased in tissue.

Effect of Polyherbal drug normalizes changes in histoarchitecture of the liver:

The liver tissue in diabetic induced rats showed dilated congested vascular space and aggregation of inflammatory cells. Evident histological alterations in Polyherbal drug treated rats showed mild aggregates of inflammatory cells. This study clearly showed the histoprotective potential of Polyherbal drug.

Effect of Polyherbal drug on insulin signalling molecules IR, IRS, Akt, SREBP, AS160 & GLUT2 mRNA expression

In high fat diet induced diabetic rats, mRNA levels of IR, IRS, Akt, SREBP & AS160 were significantly decreased, GLUT2 were significantly increased. Oral effective dose of Polyherbal drug (250 mg/kg b.wt) significantly restored the altered levels of the mRNA to that of the normal range. Control rats treated with polyherbal drug did not show any significant change in the mRNA levels.

Effect of Polyherbal drug on IR, IRS, Akt, SREBP, AS160 & GLUT2 Protein expression

IR, IRS, Akt, SREBP, AS160 & GLUT2 were significantly increased in high fat diet induced diabetic rats. Polyherbal drug aqueous treatment significantly decreased the proteins in liver of diabetes-induced rats. Normal rats treated with Polyherbal drug did not show any change in IR, IRS, Akt, SREBP, AS160 & GLUT2.

SUMMARY AND CONCLUSION:

The scientific basis for the statement that the plants and their active constituents play an important role in the prevention of chronic and degenerative diseases especially Diabetes mellitus, cardio vascular disease and carcinogenesis is continuously advancing. In recent times, the concerns over the serious adverse effects of conventional drugs and the movement towards a more natural living has brought about a resurgence in the use of herbal products. The mixture of several crude extracts, when used in formulation, enhance the beneficial effects through synergistic amplification and diminishing any possible adverse effects and offer advantage over a single isolated ingredient (Koshimizu *et al*., 1988; Williamson, 2001). Current antidiabetic drugs used in the treatment of diabetes mellitus are not consistently effective and produce adverse side effects. In recent years, the focus on identification of plant products with antidiabetic and antioxidant properties with minimal side effects has increased. Hence, in the present study Polyherbal drug a phytochemical combination consisting flowers of *Cassia auriculata*, leaves of *Gymnema sylvestre*, seeds of *Trigonella foenum graecum,* seeds of *Syzygium cumini* and bark of *cinnamomum zeylanicum* were formulated and evaluated for the first time with a view to potentiate more intense antidiabetic property. The therapeutic efficacy of Polyherbal drug was studied in high fat diet induced type 2 diabetic rats. The findings of the study shows, the Polyherbal drug have strong anti-diabetic potential in high fat diet induced type 2 diabetic rats as evidenced by increasing the body weight, antioxidant enzymes and decreasing hyperglycemia, insulin resistance, hyperlipidemia and oxidative stress in diabetic rats and also significantly altered the mRNA (IR, IRS, Akt, SREBP, AS160 & GLUT2) and protein (IR, IRS, Akt, SREBP, AS160 & GLUT2) expression in high fat diet induced type 2 diabetic rats. These findings suggested the antihyperglycemic potential of Polyherbal drug in type 2 diabetes.

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